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Regular paper

## Photosystem II solubilizes as a monomer by mild detergent treatment of unstacked thylakoid membranes\*

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**Key words:**  $\alpha$ -dodecylmaltoside, electron microscopy, grana, Photosystem II

### Abstract

We studied the aggregation state of Photosystem II in stacked and unstacked thylakoid membranes from spinach after a quick and mild solubilization with the non-ionic detergent *n*-dodecyl- $\alpha$ ,D-maltoside, followed by analysis by diode-array-assisted gel filtration chromatography and electron microscopy. The results suggest that Photosystem II (PS II) isolates either as a paired, appressed membrane fragment or as a dimeric PS II–LHC II supercomplex upon mild solubilization of stacked thylakoid membranes or PS II grana membranes, but predominantly as a core monomer upon mild solubilization of unstacked thylakoid membranes. Analysis of paired grana membrane fragments reveals that the number of PS II dimers is strongly reduced in single membranes at the margins of the grana membrane fragments. We suggest that unstacking of thylakoid membranes results in a spontaneous disintegration of the PS II–LHC II supercomplexes into separated PS II core monomers and peripheral light-harvesting complexes.

**Abbreviations:**  $\alpha$ -DM – *n*-dodecyl- $\alpha$ ,D-maltoside; BBY – PS II membranes prepared according to Berthold et al. (1981);  $\beta$ -DM – *n*-dodecyl- $\beta$ ,D-maltoside; C – PS II core monomer; Chl – chlorophyll; EM – electron microscopy; L – loosely bound trimeric LHC II; LHC II – light-harvesting complex II; M – moderately bound trimeric LHC II; MSP – manganese-stabilizing-protein; PS I – Photosystem I; PS II – Photosystem II; S – strongly-bound trimeric LHC II

### Introduction

Photosystem II (PS II) is a large supramolecular pigment–protein complex embedded in the thylakoid membranes of green plants, algae and cyanobacteria. The complex consists of more than 25 subunits, many of which are intrinsically bound to the thylakoid membranes (Ghanotakis and Yocum 1990; Hankamer et al. 2001; van Amerongen and Dekker 2002). Some subunits are involved in the capturing of solar energy and the regulation of the energy flow; others are dir-

ectly or indirectly involved in the oxidation of water to molecular oxygen.

The organization of the thylakoid membranes differs significantly in the various organisms performing oxygenic photosynthesis. Cyanobacteria, for instance, contain a rather simple membrane structure, in which, at least under the so-called ‘state 1’ conditions (light conditions under which PS I is preferentially excited), PS II is organized in rows of dimers (e.g., Bald et al. 1986). At the cytoplasmic site of each dimer, a phycobilisome is connected, which captures light in the green region of the spectrum. The thylakoid membranes of green plant chloroplasts, on the other hand, consist of two clearly distinct types of membrane do-

\* Dedicated to Prof. Charles F. Yocum on the occasion of his 60th birthday

mains (Staehelin and van der Staay 1996). One type is formed by cylindrical stacks of appressed thylakoids, known as grana, while the other, the so-called stroma membranes, appear as flattened tubules, interconnecting the grana stacks. The stacking is mediated by the presence of divalent cations and leads to a concentration in the grana of PS II and its associated light-harvesting antenna, which in these organisms is formed by a group of chlorophyll *a/b* binding proteins generally referred to as LHC II. PS I and the ATP synthase complex are predominantly restricted to the non-stacked areas of the thylakoid membranes, whereas the cytochrome *b<sub>6</sub>f* complex is probably more or less evenly distributed over the stacked and non-stacked parts of the thylakoid membranes. The stacking also allows a quick purification of the grana membranes by detergent incubation and a few centrifugation steps, leaving PS II in a relatively native state (Berthold et al. 1981). The organization of the core of PS II in such preparations, generally referred to as BBY after the authors of the procedure, is generally believed to be identical to that in cyanobacteria (e.g. Peter and Thornber 1991b; Boekema et al. 1995).

Within the last decade, impressive progress has been made on the structure and organization of PS II. The structure of the dimeric PS II core complex from the cyanobacterium *Synechococcus elongatus* is known at a resolution of 3.8 Å (Zouni et al. 2001), while the structure of trimeric LHC II from pea is known at 3.4 Å parallel to the plane of the trimer (which is also the plane of the 2-D crystals and the plane of the membrane), but 4.4–4.9 Å perpendicular to this plane (Kühlbrandt et al. 1994). In addition, a number of PS II–LHC II supercomplexes have been characterized at a resolution of about 20 Å (e.g. Boekema et al. 1995, 1999a, b; Nield et al. 2000), by which it was shown how the monomeric and some of the trimeric peripheral LHC II complexes are connected to the PS II core dimer.

A number of reports have suggested that the dimeric aggregation state of the PS II core complex in grana membranes can be stabilized by protein phosphorylation (Kruse et al. 1997), the binding of phosphatidylglycerol (Kruse et al. 2000), the presence of the PsbW protein (Shi et al. 2000) and the binding of the manganese-stabilizing protein (MSP), the extrinsic 33 kDa protein involved in oxygen evolution (Boekema et al. 2000a). A detailed understanding of the way by which the PS II core dimer is stabilized is important, because monomerization of the PS II core dimers and migration of PS II between the granal

and stromal parts of the thylakoid membranes form essential ingredients of the physiologically relevant photodamage and repair cycle of PS II (see reviews by Aro et al. 1993; Wollman et al. 1999; Choquet and Vallon 2000).

In this paper we report experiments that emphasize the role of stacking in the stabilization of the PS II core dimers. We show that the aggregation state of PS II particles solubilized by a short treatment of membranes with the very mild detergent *n*-dodecyl- $\alpha$ ,D-maltoside ( $\alpha$ -DM) strongly depends on the state of the membranes. Detergent treatment of stacked thylakoid or BBY membranes usually gives rise to PS II–LHC II supercomplexes of varying size, just as treatment of unstacked BBY membranes, but a very similar treatment of unstacked thylakoid membranes leads almost exclusively to monomeric PS II core complexes without attached peripheral light-harvesting complexes. We also analyse electron micrographs of stacked membrane fragments obtained by  $\alpha$ -DM treatment (van Roon et al. 2000), and show that dimeric PS II is strongly depleted in the single-layered parts at the margins of these membranes.

## Materials and methods

Stacked thylakoid membranes were prepared from fresh, dark-adapted spinach leaves, obtained from local shops. The leaves were depetiolated and ground in a blender in a buffer containing 50 mM Hepes (pH 7.5), 0.4 M NaCl, 2.0 mM MgCl<sub>2</sub>, 1 mM EDTA and 2.0 mg/ml bovine serum albumin, washed in a buffer containing 50 mM Hepes pH 7.5, 0.15 M NaCl and 4 mM MgCl<sub>2</sub>, and washed twice in a buffer containing 20 mM BisTris pH 6.5 and 5 mM MgCl<sub>2</sub>. Unstacked thylakoid membranes were prepared as above, but MgCl<sub>2</sub> was omitted from all buffers, while the BisTris wash buffer also contained 4 mM EDTA. Electron microscopy was used to verify that the isolated membranes were stacked and unstacked, respectively, after the above-mentioned preparation protocols. PS II membranes were isolated from freshly prepared spinach thylakoids according to Berthold et al. (1981) with minor modifications and washed twice in a buffer containing 20 mM BisTris pH 6.5 and 4 mM EDTA.

The stacked or unstacked thylakoid or BBY membranes were solubilized during 1 min with  $\alpha$ -DM or  $\beta$ -DM in the appropriate wash buffers at concentrations described in the text, and centrifuged for 3 min at 9000 rpm in an Eppendorf table centrifuge, after

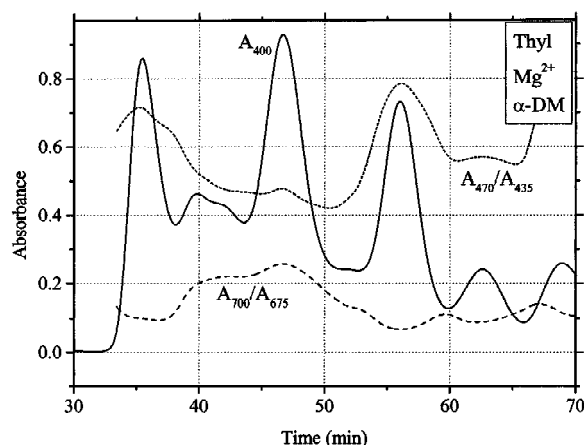


Figure 1. FPLC gel filtration chromatogram (full line) recorded at 400 nm of stacked thylakoid membranes from spinach, solubilized with  $\alpha$ -DM. The chromatography was carried out with two Superdex 200 HR 10/30 columns connected in series. The chromatogram is plotted together with the  $A_{700}/A_{675}$  ratio (dashed line) and the  $A_{470}/A_{435}$  ratio (dotted line) to get information on the relative contents of the long-wavelength antenna chlorophylls and Chl *b* + carotenoids, respectively. The values on the y-axis are the real values of these ratios and of the absorbance at 400 nm. The flow rate was 25 ml/h.

which the supernatant was pushed through a  $0.45\ \mu\text{m}$  filter to remove large fragments. The solubilized fractions were subjected to gel filtration chromatography as described by Boekema et al. (2001) with two Superdex 200 HR 10/30 columns (Pharmacia) connected in series, with 20 mM BisTris pH 6.5, 5 mM  $\text{MgCl}_2$  and 0.03%  $\alpha$ -DM as mobile phase and at a flow rate of 25 ml/h. The chromatography was performed at room temperature and monitored with a Waters 990 diode array detector.

Transmission electron microscopy was performed with a Philips CM10 electron microscope at  $52\,000\times$  magnification. Negatively stained specimens were prepared with a 2% solution of uranyl acetate on glow-discharged carbon-coated copper grids as described by Boekema et al. (2000b).

## Results

### $\alpha$ -DM treatment of stacked thylakoid membranes

Incubation of thylakoid membranes from spinach at a chlorophyll concentration of 1.4 mg/ml with 1.2%  $\alpha$ -DM usually resulted in a quick but partial 'solubilization' of the membranes, even at  $4\ ^\circ\text{C}$ . We note that small membrane fragments can also be included in the mixture of solubilized components, as long as

they are not much larger than about  $0.45\ \mu\text{m}$ , the size of the filter through which the 'solubilized' material was passed before it was subjected to the gel filtration chromatography. Figure 1 (solid line) shows a typical chromatogram of the double-column procedure, recorded at 400 nm (adapted from Boekema et al. 2001). As in the case of the single-column procedure (van Roon et al. 2000), several fractions are observed, which are, however, much better separated.

First indications of the identity of the various fractions were obtained by the simultaneously recorded ratios of the absorbances at 470 and 435 nm (dotted line) and at 700 and 675 nm (dashed line). The  $A_{470}/A_{435}$  ratio roughly monitors the ratio of chlorophyll *b* and chlorophyll *a* (and, to some extent, also the carotenoid content), whereas the  $A_{700}/A_{675}$  ratio monitors the relative content of long-wavelength absorbing chlorophylls, which are predominantly present in PS I (van Grondelle et al. 1994).

Both ratios vary quite considerably during chromatography (Figure 1). The first main fraction elutes at 35.5 min and can be attributed to PS II grana membrane fractions, in line with the low  $A_{700}/A_{675}$  ratio and the high  $A_{470}/A_{435}$  ratio. Further biochemical and electron microscopic evidence has been presented by van Roon et al. (2000) and Boekema et al. (2000b). An important conclusion was that the membrane fragments had an average diameter of 360 nm, which is close to the diameter of the grana in intact chloroplasts. The fractions at 40–42 and 47 min are characterized by a high  $A_{700}/A_{675}$  ratio and a low  $A_{470}/A_{435}$  ratio, and have been attributed to oligomeric and monomeric PS I-200 particles, respectively, on the basis of immunological and electron microscopic analyses (Boekema et al. 2001). The fractions at 56, 62 and 69 min consist primarily of trimeric LHC II (high  $A_{470}/A_{435}$  ratio), minor LHC II proteins (intermediate  $A_{470}/A_{435}$  ratio and low  $A_{700}/A_{675}$  ratio) and free pigments, respectively.

These results suggest that the  $\alpha$ -DM treatment had kept the grana in a relatively intact state as paired membranes, similar to the Triton X-100 treatment used in the procedure to obtain BBY preparations (Berthold et al. 1981), and that all other components [stroma lamellae, margins and end membranes; e.g., Albertsson (2001)] were effectively solubilized. Thus, almost all PS II occurred in the grana membrane fragments and only a minor part of PS II was directly solubilized (see also van Roon et al. 2000). A significant part of PS II in the grana membrane fragments occurred in semi-regular arrays, in which

the so-called  $C_2S_2M$  supercomplex (a dimeric PS II core complex surrounded by five monomeric and three trimeric LHC II complexes) was shown to be the basic building block (Boekema et al. 2000b). We note that  $\alpha$ -DM treatment of thylakoid membranes from *Arabidopsis thaliana* generally resulted in destabilized grana membranes and that a number of different types of PS II–LHC II supercomplexes could be observed, among which many  $C_2S_2M_2$  supercomplexes (Yakushevska et al. 2001). With lower  $\alpha$ -DM concentrations, however, grana membranes could be isolated with the  $C_2S_2M_2$  supercomplex as repeating unit of semi-regular arrays (Yakushevska et al. 2001). It should be emphasized that  $\beta$ -DM treatment of stacked thylakoid membranes from spinach resulted in a complete solubilization of the thylakoid membranes and allowed a quick and convenient purification of functionally competent  $C_2S_2$  supercomplexes (Eshaghi et al. 1999, 2000).

The general conclusion from these and other observations is that PS II usually isolates as a PS II–LHC II supercomplex upon mild detergent treatment of stacked thylakoid membranes. In these supercomplexes, PS II is organized as a dimer to which up to three monomeric LHC II proteins (the CP29, CP26 and CP24 proteins) and also up to three trimeric LHC II complexes (at the S, M and L binding sites; Boekema et al. 1999a, b) are attached at each half. The same supercomplexes also form the basic unit of regular arrays in grana membranes (Boekema et al. 2000b; Yakushevska et al. 2001), from which we conclude that PS II occurs predominantly as a dimer in the grana membranes and that the  $C_2S_2$ ,  $C_2S_2M$  or  $C_2S_2M_2$  PS II–LHC II supercomplex represents the basic building block of PS II in these membranes.

#### $\alpha$ -DM treatment of unstacked thylakoid membranes

Figure 2 shows that  $\alpha$ -DM treatment of unstacked thylakoid membranes results in a very different gel filtration chromatogram compared to the corresponding experiment with stacked membranes (Figure 1). Before going into detail, we note that the elution times differ slightly between Figures 1 and 2, because one of the two columns had to be replaced for the experiments in Figure 2. The experiments shown in Figures 3 and 4 (see below) were carried out by the same set of columns as in Figure 2, and in these chromatograms the error in the retention times of all corresponding fractions is less than 1 min. We also note that the  $A_{470}/A_{435}$  ratio is not reliable around 36 and 54 mins

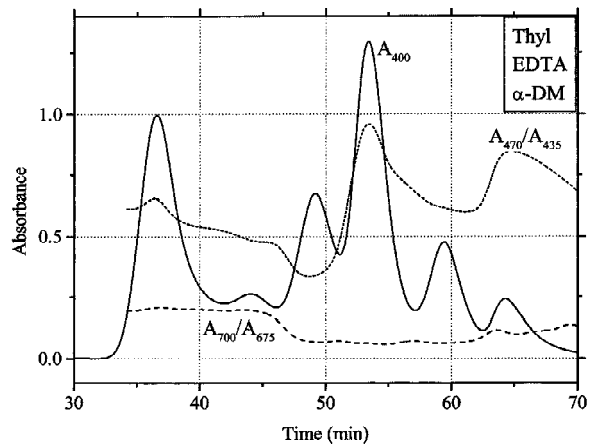


Figure 2. FPLC gel filtration chromatography of  $\alpha$ -DM solubilized, unstacked thylakoid membranes from spinach, carried out as in Figure 1. The values of the ratios are not reliable around the peaks at 36 and 54 mins because of too much absorption.

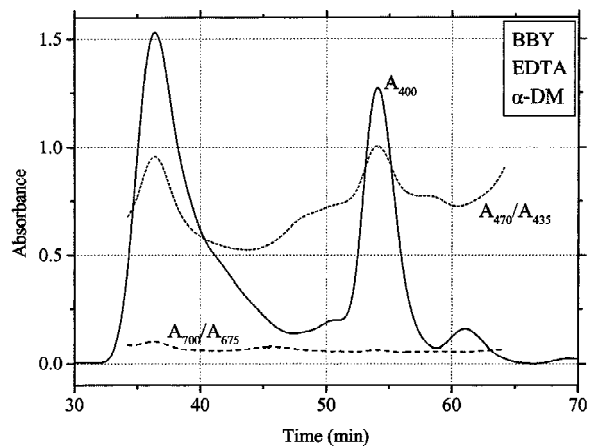


Figure 3. FPLC gel filtration chromatography of  $\alpha$ -DM-solubilized, unstacked BBY preparations from spinach, carried out as in Figure 1.

in Figure 2, because the absorption was too large at these wavelengths.

The first (main) and second (minor) peak of the chromatogram in Figure 2 appear at 36.5 and 44 min and are characterized by a high  $A_{700}/A_{675}$  ratio of about 0.21 and an intermediate  $A_{470}/A_{435}$  ratio, which suggests that these fractions are dominated by aggregates and monomers of PS I-200, respectively. The main peak at 49 min has low  $A_{700}/A_{675}$  and  $A_{470}/A_{435}$  ratios, values typical for the PS II core complex. Thus, the main difference from the elution pattern of  $\alpha$ -DM solubilized stacked thylakoid membranes (Figure 1) is that PS II elutes as a much smaller entity after unstacking. The fractions at 53.5, 59 and 64

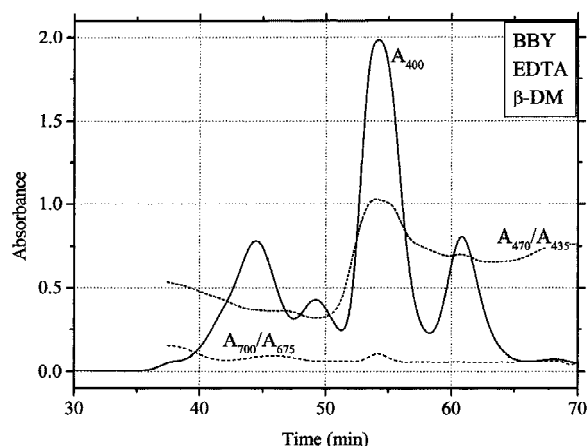


Figure 4. FPLC gel filtration chromatography of  $\beta$ -DM-solubilized, unstacked BBY preparations from spinach, carried out as in Figure 1.

min can be attributed to trimeric LHC II, monomeric LHC II and free pigments, respectively, similar to the solubilization of stacked membranes (see above).

In order to see whether the unstacking and/or the addition of EDTA itself has specific effects on the aggregation state of PS II, we solubilized BBY membranes prepared in the same EDTA-containing buffer as used for the solubilization of the unstacked thylakoid membranes with either  $\alpha$ -DM or  $\beta$ -DM, and recorded the gel filtration chromatograms under the same buffer and detergent conditions as Figures 1 and 2. The results are shown for  $\alpha$ -DM-solubilization in Figure 3 and  $\beta$ -DM-solubilization in Figure 4. Both chromatograms are qualitatively similar to those reported by Boekema et al. (1998), despite the fact that in the earlier paper EDTA was not used, the gel filtration was carried out with a single column and about 5 times lower chlorophyll and detergent concentrations were used for the solubilization. The various ratios (Figure 4) and spectra (not shown) of the  $\beta$ -DM-solubilized material suggest that the fractions at 40–42, 44.5 and 49 min must be attributed to PS II–LHC II supercomplexes, PS II core dimers and PS II core monomers, respectively. Solubilization with  $\alpha$ -DM (Figure 3) revealed many membrane fragments (at 36.5 min) and PS II–LHC II supercomplexes (around 42 mins), but virtually no PS II core dimers (expected at 44.5 min) and PS II core monomers (expected at 49 min). The small peak at 50.5 min had a high  $A_{470}/A_{435}$  ratio and a very different spectrum from that of the PS II core complex. The spectrum and size of this minor component are consistent with those of the supercom-

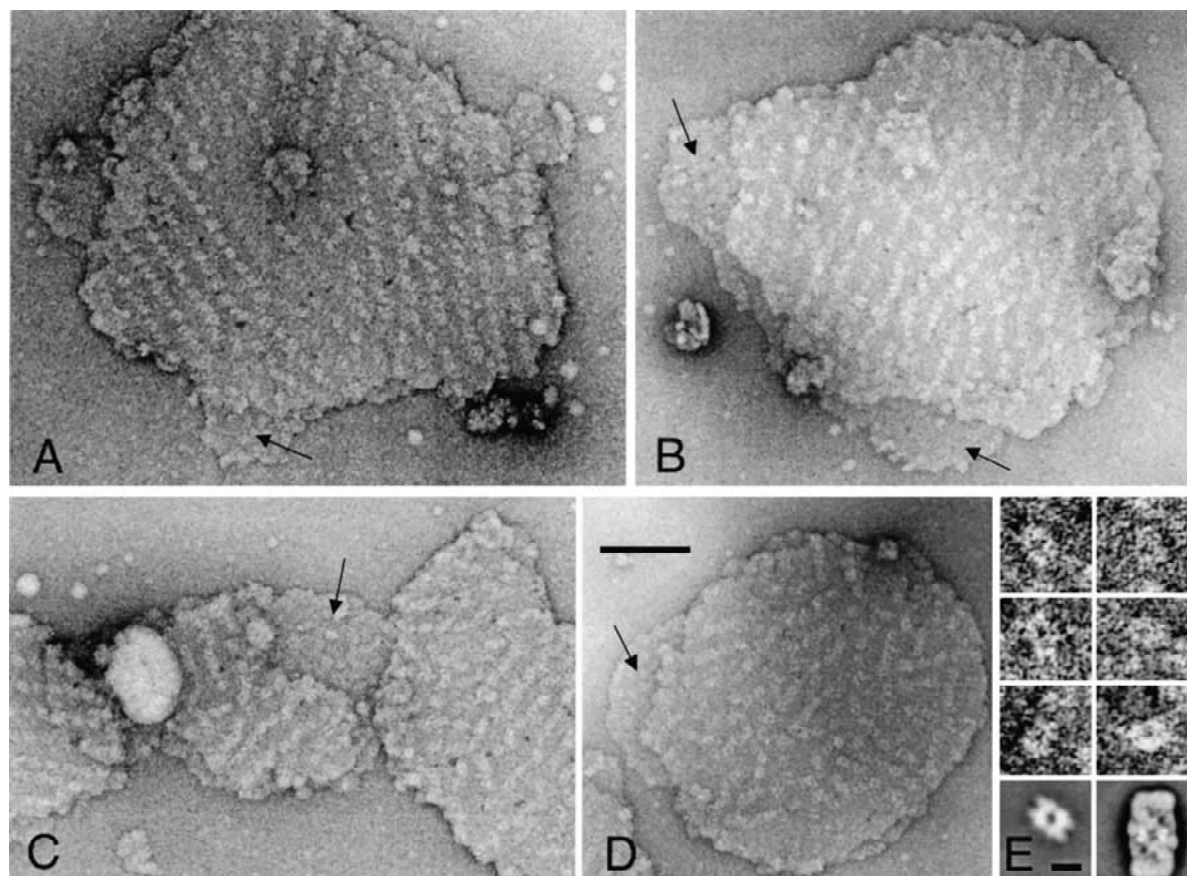
plex of trimeric LHC II, CP29 and CP24 (Peter and Thornber 1991a; Bassi and Dainese 1992).

Comparison of the results presented in Figures 3 and 4 with those in Boekema et al. (1998) indicates that unstacking of BBY membranes by omitting divalent cations and adding EDTA does not have a significant effect on the aggregation state of PS II, and PS II occurs predominantly as a dimeric supercomplex under these conditions, though the amount of dimers with attached LHC II complexes is larger after  $\alpha$ -DM solubilization than after  $\beta$ -DM solubilization. Even unstacking of Tris- or salt-washed PS II membranes resulted in many PS II–LHC II supercomplexes after  $\alpha$ -DM solubilization (Boekema et al. 2000a). After unstacking of thylakoid membranes, however, almost all PS II elutes at 49 min (Figure 4), which is typical for the monomeric aggregation state (Figure 2), despite the very similar solubilization conditions. The chromatogram also shows that almost no PS II occurs in a larger unit, because the small absorption at 44.5 and 40–42 min (where the PS II core dimers and PS II–LHC II supercomplexes are expected) are clearly dominated by PS I.

The general conclusion from these observations is that PS II isolates as a monomer upon mild detergent treatment of unstacked thylakoid membranes, and as a dimer or supercomplex upon mild detergent treatment of unstacked BBY membranes. These results are in line with those of Bassi et al. (1995) who showed by non-denaturing Deriphat-PAGE that PS II is monomeric in stroma lamellae and dimeric in grana.

#### *Electron microscopy of grana membrane fragments*

The results presented above do not prove that PS II is monomeric in unstacked membranes, but only that it isolates as a monomer upon mild solubilization procedures. In order to get more insight into organization of PS II in the grana membranes prepared after an  $\alpha$ -DM treatment of stacked thylakoid membranes from spinach (van Roon et al. 2000), we show EM micrographs of four of such membranes in Figures 5A–D. It is clear that most of these membranes consist of two membranes stacked on top of each other, but in all membranes there are small single-layered parts at the margins (see arrows). These parts can either be remnants of the original stroma membranes, or they can originate from the treatment used to prepare these membranes. The distribution and size of the particles in the single-layered parts differ rather significantly from those in the double-layered parts. In the latter



**Figure 5.** (A–D) Electron micrographs of paired grana membrane fragments from spinach, negatively stained with 2% uranyl acetate. The most prominent stain-excluding subunits, which presumably originate from the extrinsic proteins attached to the core parts of PS II, are predominantly located in paired membranes and are virtually absent in single-layered parts (arrows). In some membranes (C, D) there is almost no ordering of PS II core dimers, while others (A, B) show a semi-crystalline lattice in which the distance between rows of PS II complexes is about 26.3 nm (Boekema et al. 2000b). The scale bar is 100 nm. (E) Images of membrane-bound PS II dimers in BBY preparations. The image on the bottom left is the average of 300 projections as in the six top images. The image on the bottom, right, is an average of 600 single-particle projections of isolated  $C_2S_2$  PS II–LHC II supercomplexes (from Boekema et al. 1999a). The magnification of the images in (E) is exactly three times higher than of those in (A–D). The scale bar is 10 nm.

regions, many structures are seen consisting of a stain-excluding rectangular shape with a stain-spot in the center. Very similar structures were observed in BBY membranes (Figure 5E), of which the average (Figure 5E, bottom left) shows a clear resemblance to the part attributed to the PS II core dimer in the  $C_2S_2$  PS II–LHC II supercomplex (Figure 5E, bottom right). The stain distribution of this part of the complex arises mainly from the extrinsic subunits and the hydrophilic loops of CP47 and CP43 of the PS II core dimer, which extend more than 5 nm from the membrane surface in the lumen (Nield et al. 2000). The much lower amount of the typical PS II core dimer structures in the single-layered parts suggests that dimeric PS II is only stable

in the stacked, double-layered parts of the thylakoid membranes.

### Discussion

The results presented here reveal that under mild ( $\alpha$ -DM) detergent solubilization conditions PS II isolates predominantly as a monomer from unstacked thylakoid membranes and as a dimeric supercomplex from stacked thylakoid membranes. These results are in line with those from the non-denaturing Deriphat-PAGE experiments of Peter and Thornber (1991a), who showed that PS II in BBY membranes fractionates into supercomplexes and PS II in unstacked

thylakoid membranes into monomers, and of Bassi et al. (1995), who showed that PS II in stroma membranes fractionates as a monomer.

It is not clear whether PS II also exists as a monomer in the unstacked parts of the thylakoid membranes. It is, in principle, possible that also in these membranes PS II is dimeric *in vivo* but monomerizes as a result of the detergent treatment. Staehelin (1976) used freeze-fracture EM to investigate the changes in size distributions and densities of particles upon unstacking and restacking of thylakoid membranes from spinach, and interpreted the results by a detachment of a part of the peripheral light-harvesting antenna upon unstacking, and not by a monomerization of PS II. However, it is hard to get an idea of the molecular origin of the particles observed in the fracture faces [see Staehelin and van der Staay (1996), for an explanation of the various fracture and surface faces]. In addition, it is not clear whether the tetrameric appearance of the so-called ES surface particles in the stacked areas is fully preserved in the unstacked areas, as stated by Staehelin (1976). We note that it was not known at the time of writing of Staehelin's paper that the tetrameric ES surface particles reflect in fact the extrinsic proteins and the hydrophilic loops of CP47 and CP43 of dimeric PS II (Seibert et al. 1987; Hankamer et al. 1997). A reinvestigation of the shape of the ES surface particles in the various membrane fractions seems therefore appropriate. In the more recent review of Staehelin and van der Staay (1996) it is discussed that PS II is indeed monomeric in unstacked membranes and dimeric in grana membranes, which would be consistent with our results presented in Figure 5 and which would suggest that the organization of particles obtained by mild detergent solubilization reflects the *in vivo* organization. A monomeric organization of PS II in unstacked membranes is also consistent with photochemical activity measurements (Boichenko 1998).

The results presented in Figures 3 and 4 indicate that PS II is organized as a dimeric PS II-LHC II complex in EDTA-treated BBY membranes. It is possible that the treatment utilized to unstack the thylakoids did not induce a separation of the two membranes in the BBY preparations, and that therefore the membranes were formally not 'unstacked'. However, several experiments have indicated that the EDTA treatment enhances the solubilizing effect of  $\alpha$ -DM, in particular when the membranes were extensively washed with detergent-free buffers before solubilization, such as the Tris and salt buffers to

obtain membranes depleted of extrinsic polypeptides (Boekema et al. 2000a). EM micrographs have indeed revealed that EDTA-treated BBY preparations consist of single membranes (not shown), though extensively washed preparations sometimes appeared to be so strongly laterally aggregated that it was not always obvious to decide whether the membranes were single or paired. Anyway, the removal of divalent cations induced a state of the membranes which was much better accessible to  $\alpha$ -DM, which would be in line with the idea that  $\alpha$ -DM should have access to the region between the membranes in a stack to perform its solubilizing action.

Another explanation for the observation that PS II is organized as a dimeric supercomplex in unstacked BBY preparations and as a monomer in unstacked thylakoids can be provided by the lipid composition of the membranes in which PS II is located. Unstacking of BBY preparations will not change the lipid composition, whereas unstacking of thylakoids will result in a redistribution of all thylakoid lipids (and other components of the thylakoid membrane), in which the equilibrium between the monomeric and supercomplex forms could be at the side of the monomers in the stroma lamellae and unstacked membranes, and at the side of the supercomplexes in the grana membranes. A spontaneous monomerization of PS II as soon as it reaches the stromal parts of the membranes will certainly be helpful for the kinetics of the repair cycle of the D1 protein (Baena-González et al. 1999), in which the migration of damaged PS II from grana to stroma and the disintegration of the supercomplexes into monomers form essential ingredients, as well as the reverse processes after the integration of a repaired D1 protein in the PS II core monomer.

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